# Editorial

# Association of Blood Pressure With Genetic Variation in *WNK* Kinases in a White European Population

Haifeng Zhang, MD; Jan A. Staessen, MD, PhD

Mutations in a recently discovered family of protein kinases are responsible for an autosomal-dominant form of inherited hypertension, known as Gordon's syndrome or pseudohypoaldosteronism type II (PHAII).[1] The phenotype also includes hyperkalemia and hyperchloremic metabolic acidosis.[1] The name of this kinase family is *WNK* (with no lysine [K]) because of the absence of a lysine in subdomain II of the enzymes.[2] *WNK1* and *WNK4*, located in human chromosomes 12 and 17, respectively, are predominantly expressed in the distal convoluted tubules and the collecting ducts of the kidney.[1]

**Article p 3423**

In vitro, wild-type *WNK4* inhibits the thiazide-sensitive sodium chloride cotransporter (NCCT)[3,4] and the renal outer medullary potassium ion channel (ROMK),[5] but increases paracellular chloride permeability.[6] Some *WNK4* mutations identified in PHAII behave as a loss of function for the NCCT inhibition[3,4,7] but as a gain of function for the inhibition of ROMK,[5,7] and further stimulate paracellular chloride transport.[6,8] These effects of *WNK4* mutations fit well with the proposed mechanisms for the development of hypertension and electrolyte abnormalities in PHAII. Furthermore, in vitro, *WNK1* counteracts the inhibition of NCCT by *WNK4*[4] and activates the serum- and glucocorticoid-inducible protein kinase (*SGK1*), which in turn stimulates the epithelial sodium channel (ENaC).[9] The observation that heterozygous *WNK1*-deficient mice had lower blood pressure than wild-type control animals further supports the role of WNK1 in blood pressure regulation.[10] The evidence from studies in patients with PHAII,[1] cell models,[3–9] and genetically engineered animals[10] makes *WNK1* and *WNK4* strong candidates possibly involved in the pathogenesis of essential hypertension. However, previous case-control[11–13] and population-based[14] studies of various single nucleotide polymorphisms (SNPs) in *WNK1* and *WNK4* yielded inconsistent results.

The study published by Tobin and colleagues[15] in this issue of *Circulation* may herald a breakthrough in unraveling the putative association between blood pressure and genetic variation in the *WNK* kinases. The authors recruited via family practitioners a family-based sample of nuclear families consisting of both parents and 2 offspring, which was representative of the general population of Leicestershire, UK. A history of hypertension was not among the inclusion criteria. The researchers employed 26-hour ambulatory monitoring to measure the blood pressure phenotypes. Compared with conventional blood pressure measurement, ambulatory monitoring is characterized by high reproducibility, is not subject to digit preference and observer bias, and avoids the so-called white coat effect, which is the transient rise in a subject's blood pressure in response to the clinical surroundings or the presence of an observer. The authors discarded the first 2 hours of each ambulatory recording to avoid any alerting response. In addition to the precision of the phenotype, Tobin and colleagues extensively genotyped 9 SNPs in *WNK1*, 8 of which were sufficient to predict the common haplotypes. They also measured 1 intronic SNP in *WNK4*, which is frequent among white Europeans. The heritability of the 24-hour systolic and diastolic blood pressure was around 65%. The authors found statistically significant associations of mean 24-hour systolic and/or diastolic blood pressures with several common SNPs and haplotypes in *WNK1*. The mean estimated effect sizes for single SNPs approximately ranged from a 2-mm Hg reduction to a 1-mm Hg increase. In contrast, there was no association between blood pressure and the SNP in *WNK4*. All findings remained consistent after adjustment for sex, age, body mass index, smoking, alcohol intake, history of hypercholesterolemia, and education level. The demonstration that common variants in *WNK1*, a gene causing a rare monogenic form of hypertension, contributes to the blood pressure variation in the general population is a genuine breakthrough.

Whereas the study by Tobin and colleagues[15] lifts a corner of the veil surrounding the *WNK* paradigm, it also leaves several questions unanswered. First, the authors did not measure serum concentration or 24-hour excretion of electrolytes, nor the circulating components of the renin-angiotensin system. *WNKs* are widely expressed in epithelia.[16,17] Their study[15] therefore does not prove with absolute certainty that the association with blood pressure was renally mediated, although the latter hypothesis is most likely in view of the previous evidence.[4,9] Second, the genetic epidemiological findings are not underpinned by in vitro studies proving functionality of the *WNK1* haplotypes in renal tubular cells. Third, the findings by Tobin et al[15] are at variance with another recent report[14] published by the same investigators.

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

From the Study Coordinating Centre, Hypertension and Cardiovascular Rehabilitation Unit, Department of Molecular and Cardiovascular Research, University of Leuven, Leuven, Belgium (H.Z., J.A.S.), and the Department of Cardiology, First Affiliated Hospital, Nanjing Medical University, Nanjing, China (H.Z.).

Correspondence to Jan A. Staessen, Study Coordinating Centre, Hypertension and Cardiovascular Rehabilitation Unit, Department of Molecular and Cardiovascular Research, Campus Gasthuisberg, University of Leuven, Herestraat 49, B-3000 Leuven, Belgium. E-mail jan.staesssen@med.kuleuven.be

**(*Circulation*. 2005;112:3371-3372.)**



***Circulation* is available at http://www.circulationaha.org**
**DOI: 10.1161/CIRCULATIONAHA.105.585737**

Indeed, family-based association tests involving 712 severely hypertensive families in the British Genetics of Hypertension (BRIGHT) Study[14] showed significantly positive associations of systolic ($z=2.241$, $P=0.025$) and diastolic ($z=1.992$, $P=0.046$) blood pressures with the rs1468326 SNP in *WNK1*, and a negative association of systolic ($z=-2.300$, $P=0.017$) blood pressure with the *WNK1* haplotype h10 AGCCTCCG. In the present study, these associations had an opposite sign and did not reach statistical significance.[15] This was also the case for the common *WNK1* haplotype h4 CACCCCCG, which was positively correlated with systolic pressure ($z=1.912$, $P=0.053$) in the BRIGHT study[14] but negatively with systolic and diastolic pressures during daytime and over 24 hours ($z\leq-2.142$; $P\leq0.032$).[15] These contradictory results require clarification. Fourth, Tobin and colleagues[15] did not investigate the possible interaction between genetic variation in the *WNK*s and salt intake in relation to blood pressure. The European Project on Genes in Hypertension[18] highlighted that phenotype–genotype relationships strongly depend on lifestyle, in particular salt intake, as reflected by the 24-hour urinary excretion of sodium. Ignoring such interaction may explain the aforementioned inconsistencies.

Irrespective of its limitations, the present study[15] is the first study that demonstrates that common genetic variants of *WNK1* contribute to blood pressure variation in a general white population. If these findings are confirmed by other epidemiological studies and if they are supported by experimental evidence for functionality, then they may have wide-ranging implications. *WNK* kinases may become novel targets for pharmaceutical intervention and lead to the development of diuretic agents without the metabolic side effects of thiazides. Moreover, further studies should address the question to what extent genetic variation in the *WNK*s influences salt sensitivity and the response to antihypertensive drugs and whether they can predict the incidence of hypertension and cardiovascular morbidity and mortality. Thus, the article by Tobin and colleagues[15] is a significant step forward, but the onward journey to the possible clinical applications of the *WNK* paradigm remains long.

## References


1. Wilson FH, Disse-Nicodeme S, Choate KA, Ishikawa K, Nelson-Williams C, Desitter I, Gunel M, Milford DV, Lipkin GW, Achard JM, Feely MP, Dussol B, Berland Y, Unwin RJ, Mayan H, Simon DB, Farfel Z, Jeunemaitre X, Lifton RP. Human hypertension caused by mutations in WNK kinases. *Science*. 2001;293:1107–1112.
2. Xu B, English JM, Wilsbacher JL, Stippec S, Goldsmith EJ, Cobb MH. WNK1, a novel mammalian serine/threonine protein kinase lacking the catalytic lysine in subdomain II. *J Biol Chem*. 2000;275:16795–16801.
3. Wilson FH, Kahle KT, Sabath E, Lalioti MD, Rapson AK, Hoover RS, Hebert SC, Gamba G, Lifton RP. Molecular pathogenesis of inherited hypertension with hyperkalemia: the Na-Cl cotransporter is inhibited by wild-type but not mutant WNK4. *Proc Natl Acad Sci U S A*. 2003;100:680–684.
4. Yang CL, Angell J, Mitchell R, Ellison DH. WNK kinases regulate thiazide-sensitive Na-Cl cotransport. *J Clin Invest*. 2003;111:1039–1045.
5. Kahle KT, Wilson FH, Leng Q, Lalioti MD, O'Connell AD, Dong K, Rapson AK, MacGregor GG, Giebisch G, Hebert SC, Lifton RP. WNK4 regulates the balance between renal NaCl reabsorption and $K^+$ secretion. *Nat Genet*. 2003;35:372–376.
6. Kahle KT, Macgregor GG, Wilson FH, Van Hoek AN, Brown D, Ardito T, Kashgarian M, Giebisch G, Hebert SC, Boulpaep EL, Lifton RP. Paracellular $Cl^-$ permeability is regulated by WNK4 kinase: insight into normal physiology and hypertension. *Proc Natl Acad Sci U S A*. 2004;101:14877–14882.
7. Golbang AP, Murthy M, Hamad A, Liu CH, Cope G, Van't Hoff W, Cuthbert A, O'Shaughnessy KM. A new kindred with pseudohypoaldosteronism type II and a novel mutation (564D>H) in the acidic motif of the WNK4 gene. *Hypertension*. 2005;46:295–300.
8. Yamauchi K, Rai T, Kobayashi K, Sohara E, Suzuki T, Itoh T, Suda S, Hayama A, Sasaki S, Uchida S. Disease-causing mutant WNK4 increases paracellular chloride permeability and phosphorylates claudins. *Proc Natl Acad Sci U S A*. 2004;101:4690–4694.
9. Xu BE, Stippec S, Chu PY, Lazrak A, Li XJ, Lee BH, English JM, Ortega B, Huang CL, Cobb MH. WNK1 activates SGK1 to regulate the epithelial sodium channel. *Proc Natl Acad Sci U S A*. 2005;102:10315–10320.
10. Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT. Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. *Proc Natl Acad Sci U S A*. 2003;100:14109–14114.
11. Erlich PM, Cui J, Chazaro I, Farrer LA, Baldwin CT, Gavras H, DeStefano AL. Genetic variants of WNK4 in whites and African Americans with hypertension. *Hypertension*. 2003;41:1191–1195.
12. Speirs HJ, Morris BJ. WNK4 intron 10 polymorphism is not associated with hypertension. *Hypertension*. 2004;43:766–768.
13. Kokubo Y, Kamide K, Inamoto N, Tanaka C, Banno M, Takiuchi S, Kawano Y, Tomoike H, Miyata T. Identification of 108 SNPs in TSC, WNK1, and WNK4 and their association with hypertension in a Japanese general population. *J Hum Genet*. 2004;49:507–515.
14. Newhouse SJ, Wallace C, Dobson R, Mein C, Pembroke J, Farrall M, Clayton D, Brown M, Samani N, Dominiczak A, Connell JM, Webster J, Lathrop GM, Caulfield M, Munroe PB. Haplotypes of the WNK1 gene associate with blood pressure variation in a severely hypertensive population from the British Genetics of Hypertension study. *Hum Mol Genet*. 2005;14:1805–1814.
15. Tobin MD, Raleigh SM, Newhouse S, Braund P, Bodycote C, Ogleby J, Cross D, Gracey J, Hayes S, Smith T, Ridge C, Caulfield M, Sheehan NA, Munroe PB, Burton PR, Samani NJ. Association of *WNK1* gene polymorphisms and haplotypes with ambulatory blood pressure in the general population. *Circulation*. 2005;112:3423–3429.
16. O'Reilly M, Marshall E, Speirs HJ, Brown RW. WNK1, a gene within a novel blood pressure control pathway, tissue-specifically generates radically different isoforms with and without a kinase domain. *J Am Soc Nephrol*. 2003;14:2447–2456.
17. Kahle KT, Gimenez I, Hassan H, Wilson FH, Wong RD, Forbush B, Aronson PS, Lifton RP. WNK4 regulates apical and basolateral $Cl^-$ flux in extrarenal epithelia. *Proc Natl Acad Sci U S A*. 2004;101:2064–2069.
18. Kuznetsova T, Staessen JA, Brand E, Cwynar M, Stolarz K, Thijs L, Tikhonoff V, Wojciechowska W, Babeanu S, Brand-Herrmann SM, Casiglia E, Filipovsky J, Grodzicki T, Nikitin Y, Peleška J, Struijker-Boudier H, Bianchi G, Kawecka-Jaszcz K; European Project On Genes in Hypertension (EPOGH) Investigators. Sodium excretion as modulator of genetic associations with cardiovascular phenotypes in the European Project on Genes in Hypertension. *J Hypertens*. In press.


KEY WORDS: Editorials ■ cardiovascular diseases ■ hypertension ■ genetics ■ blood pressure

# Association of *WNK1* Gene Polymorphisms and Haplotypes With Ambulatory Blood Pressure in the General Population

Martin D. Tobin, PhD; Stuart M. Raleigh, PhD; Stephen Newhouse, MSc; Peter Braund, MSc; Clare Bodycote, BSc; Jenny Ogleby, BSc; Deborah Cross, PhD; Jay Gracey, BA; Saija Hayes, BSc; Terry Smith, RGN; Cathy Ridge, BA; Mark Caulfield, FRCP; Nuala A. Sheehan, PhD; Patricia B. Munroe, PhD; Paul R. Burton, MD; Nilesh J. Samani, MD

***Background***—Blood pressure (BP) is a heritable trait of major public health concern. The *WNK1* and *WNK4* genes, which encode proteins in the WNK family of serine-threonine kinases, are involved in renal electrolyte homeostasis. Mutations in the *WNK1* and *WNK4* genes cause a rare monogenic hypertensive syndrome, pseudohypoaldosteronism type II. We investigated whether polymorphisms in these *WNK* genes influence BP in the general population.

***Methods and Results***—Associations between 9 single-nucleotide polymorphisms (SNPs) in *WNK1* and 1 in *WNK4* with ambulatory BP were studied in a population-based sample of 996 subjects from 250 white European families. The heritability estimates of mean 24-hour systolic BP (SBP) and diastolic BP (DBP) were 63.4% and 67.9%, respectively. We found statistically significant ($P<0.05$) associations of several common SNPs and haplotypes in *WNK1* with mean 24-hour SBP and/or DBP. The minor allele (*C*) of rs880054, with a frequency of 44%, reduced mean 24-hour SBP and DBP by 1.37 (95% confidence interval, −2.45 to −0.23) and 1.14 (95% confidence interval, −1.93 to −0.38) mm Hg, respectively, per copy of the allele.

***Conclusions***—Common variants in *WNK1* contribute to BP variation in the general population. This study shows that a gene causing a rare monogenic form of hypertension also plays a significant role in BP regulation in the general population. The findings provide a basis to identify functional variants of *WNK1,* elucidate any interactions of these variants with dietary intake or with response to antihypertensive drugs, and determine their impact on cardiovascular morbidity and mortality. (***Circulation*. 2005;112:3423-3429.**)

**Key Words:** blood pressure ■ genetics ■ hypertension ■ kidney ■ risk factors

Blood pressure (BP) is a key determinant of cardiovascular health.[1,2] Familial aggregation of BP has long been recognized, and estimates of the heritability of systolic (SBP) and diastolic (DBP) BP have exceeded 50%.[3,4] The identification of genes involved in BP regulation, by improving knowledge of the relevant biology, should facilitate advances in treatment and control of BP. However, BP is a complex trait, and genetic studies into its etiology are constrained by the small effect sizes of the individual genetic variants, imprecise measures of the phenotype, and low-power approaches to study design and analysis.

**Editorial p 3371**

Studies of monogenic syndromes have provided important insights into a number of mechanisms underlying BP regulation.[5] Although these provide evidence for a causal relation between gene and disease, none of these genes have yet been shown to directly affect BP in the general population.[5] Recently, mutations in the *WNK1* and *WNK4* genes, which encode proteins in the WNK ("with no lysine" [K]) family of serine-threonine kinases, have been shown to cause pseudohypoaldosteronism type II (PHAII, or Gordon's syndrome), an autosomal-dominant condition characterized by hypertension and hyperkalaemia.[6] The WNK1 and WNK4 proteins localize to distal nephrons, WNK1 normally inhibiting WNK4 and WNK4 normally inhibiting the Na-Cl cotransporter in the apical membrane of epithelial cells lining the distal convoluted tubule.[7,8] Thus, "gain-in-function" mutations in *WNK1* or "loss-of-function" mutations in *WNK4* result in PHAII that involves Na-Cl cotransporter overactivity.[7,8] The Na-Cl cotransporter is sensitive to thiazide diuretics, and patients with PHAII exhibit an unusually large BP fall in response to thiazides.[9]

Received April 12, 2005; revision received July 26, 2005; accepted August 22, 2005.

From the Department of Health Sciences (M.D.T., N.A.S., P.R.B.), University of Leicester; the Department of Cardiovascular Sciences (S.M.R., P.B., C.B., J.O., D.C., J.G., S.H., T.S., C.R., N.J.S.), University of Leicester, Glenfield Hospital; and the Department of Genetics (M.D.T., N.A.S., P.R.B.), University of Leicester, Leicester; and the Department of Clinical Pharmacology and Barts and the London Genome Centre (S.N., M.C., P.B.M.), Barts and the London School of Medicine, London, England.

**The online-only Data Supplement can be found at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.105.555474/DC1.**

Correspondence to Prof Nilesh J. Samani, Department of Cardiovascular Sciences, University of Leicester, Clinical Sciences Wing, Glenfield Hospital, Groby Road, Leicester LE3 9QP, UK. E-mail njs@le.ac.uk



*Circulation* **is available at http://www.circulationaha.org** **DOI: 10.1161/CIRCULATIONAHA.105.555474**

*WNK1* spans 156 kb of genomic DNA, with at least 28 exons producing multiple transcripts owing to alternative splicing, and is highly polymorphic, with >100 validated single-nucleotide polymorphisms (SNPs) (dbSNP, build 124; available at http://www.ncbi.nlm.nih.gov/projects/SNP/; last accessed February 11, 2005). *WNK4* spans 16 kb of genomic DNA and 19 exons and, in white European subjects, is much less polymorphic, with only 1 common SNP reported (1156666G→A).[10]

A few studies have examined the association of polymorphisms in *WNK1* and/or *WNK4* with the risk of hypertension.[10–13] A study in Japanese subjects found a nominal association with hypertension and 1 *WNK4* SNP (C14717T).[11] Although an association between another *WNK4* SNP (1156666G→A) and hypertension has been reported in white American subjects,[10] this finding was not replicated in a study of white Australian subjects,[12] but both studies were small. Recently, the BRIGHT study provided evidence supporting a possible association between a *WNK1* SNP (rs1468326) and the severity of hypertension in a family study of extremely hypertensive white European subjects.[13] However, no study has yet examined whether common variants in *WNK1* or *WNK4* are associated with BP regulation in a population-based sample that has not been selected for the presence or absence of hypertension.

The main objective of our study was to investigate the association between common SNPs in *WNK1* with BP in a population-based sample. Because BP measured at a single time is subject to transient variation and because BP exhibits circadian variations, we measured ambulatory BP for 24 hours to characterize it more precisely and to maximize the power to detect genetic determinants with a modest effect on BP. For completeness, we also included in our investigation the single *WNK4* SNP observed in white European populations.[10]

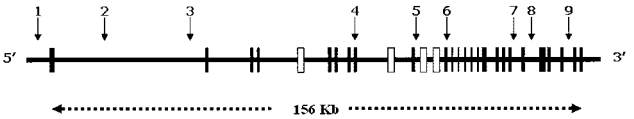
# Methods

## Subjects and Phenotyping

We studied 1005 white European subjects from 252 nuclear families recruited from the general population in the ongoing GRAPHIC (Genetic Regulation of Arterial Pressure of Humans in the Community) study.

Families were included if both parents aged 40 to 60 years and 2 offspring ≥18 years wished to participate. Families were recruited by writing to women aged 40 to 59 years who had registered with participating family practitioners in Leicestershire, England, inviting them and their families to take part. Subjects were excluded if they had renal disease or a comorbidity that affected accurate BP measurement. There was no preferential selection based on history of hypertension. Interviews by research nurses consisted of a detailed history and examination, including clinic BP and collection of blood samples. The Leicestershire Research Ethics Committee approved the study, and all subjects provided written, informed consent.

Ambulatory BP was measured with a Spacelabs 90207 monitor (Spacelabs) for 26 hours. The first 2 hours of each record were discarded to avoid any alerting response. The ambulatory monitor recorded BP at 30-minute intervals between 8 AM and 9:59 PM ("daytime") and at 1-hour intervals between 10 PM and 7:59 AM ("nighttime"). If ambulatory BP profiles were <80% complete, they were repeated. We summarized the ambulatory BP data, weighting each time period proportional to its length. The 6 phenotypic outcomes on which our analyses focused were the time-weighted-means of SBP and DBP for 24 hours, during the daytime, and during the nighttime.




Structure of the human *WNK1* gene and location of the SNPs analyzed. Exons are shown as vertical lines. Alternatively spliced exons are unfilled. The locations of the SNPs typed are indicated by arrows and are as follows: (1) rs1468326, (2) rs2369402, (3) rs765250, (4) rs2286007, (5) rs880054, (6) rs956868, (7) rs953361, (8) rs2301880, and (9) rs2286028.

Further details of recruitment and phenotyping, including response rates and measurement of clinic BP, are included in the online-only Data Supplement.

## Genotyping and Quality Assurance

We genotyped 9 *WNK1* SNPs (the Figure). The choice of SNP was informed by the results of a prior analysis of *WNK1* described elsewhere.[13] In brief, 19 SNPs spanning *WNK1* at ≈10 kb and including all nonsynonymous coding SNPs and the SNPs immediately 5′ and 3′ of the gene were typed in a separate population of 100 families.[13] The 9 SNPs were chosen in an initial tag SNP (tSNP) selection by eye. In a more detailed analysis with established criteria,[14] 8 of these SNPs (excluding rs2301880) were found to be sufficient to predict common *WNK1* haplotypes (minimum $R_h^2$=100%, $R_s^2$=94%) with >90% power.[13] However, we present data on all typed SNPs, including rs2301880. The *WNK4* (1156666G→A) SNP[10] was also typed. Genotyping for the SNPs was done by fluorescent allelic discrimination with the ABI Prism 7900HT sequence detector system (Applera). Details are given in the Data Supplement.

To assist in the identification of misspecified family relationships, we also genotyped 3 highly polymorphic microsatellite markers, d19s220, d3s1267, and d4s412, from the ABI Prism linkage mapping set v2.5-MD10 (PE, Applied Biosystems). Details are given in the Data Supplement. We checked for misspecified family relationships with the PedCheck program.[15] Simulation analysis in 1000 families showed that the 3 microsatellites provided >99.3% power to detect any mendelian inconsistency. Two complete families and 1 individual (total of 9 subjects) were excluded after showing mendelian inconsistencies. In the remaining 996 subjects from 250 families, using PedCheck we then searched for mendelian inconsistencies in the SNP data attributable to genotyping error. The SNP data that were inconsistent with mendelian inheritance (13 observations) were coded as missing.

## Statistical Analysis

Departure from Hardy-Weinberg equilibrium was tested with a $\chi^2$ test on parental SNP data. We estimated |D'| and $R^2$ measures of linkange disequilibrium between pairs of SNPs with the JLIN program.[16]

Estimates of variance components, heritability, and the effects of individual SNPs were obtained by fitting generalized linear mixed models (GLMMs) using Gibbs sampling in WinBUGS.[17,18] These models deal appropriately with the correlation of traits, genotypes, and environmental exposures within families.[17,18] A censored normal approach[19] was used to adjust for the effect of antihypertensive therapy. A GLMM was fitted to estimate narrow-sense heritability for each ambulatory BP phenotype, including age and sex (but no genes) as covariates. The SNP covariates were then included in the model, 1 at a time, and the effect of each SNP was estimated under an additive genetic model. Although the GLMMs were fitted with a Bayesian approach, flat prior distributions were used throughout, and inferences are reported as probability values and 95% confidence intervals (CIs).[20]

Using the 8 tSNPs, we also undertook a test of association of *WNK1* haplotypes with BP phenotypes in the presence of linkage by

**TABLE 1. Characteristics of the Study Participants**

| Variable | Parents (n=499) | Offspring (n=497) | All Subjects (N=996) |
|---|---|---|---|
| General characteristics | | | |
| Male/female, n/n | 250/249 | 250/247 | 500/496 |
| Age, median (range), y | 53.0 (40–60) | 24.0 (18–41) | 41.5 (18–60) |
| History of hypertension, n (%) | 117 (23.4) | 19 (3.8) | 136 (13.7) |
| Current antihypertensive treatment, n (%) | 62 (12.4) | 1 (0.2) | 63 (6.3) |
| Current smoker, n (%) | 64 (12.8) | 141 (28.4) | 205 (20.6) |
| Weekly alcohol intake ≥21 units (women) and ≥28 units (men),† n (%) | 95 (19.2) | 65 (13.1) | 160 (16.2) |
| Body mass index, mean (SD), kg/m$^2$ | 27.5 (4.2) | 24.8 (4.9) | 26.1 (4.8) |
| History of high cholesterol | 38 (7.6) | 5 (1.0) | 43 (4.3) |
| Diabetes, n (%) | 8 (1.6) | 1 (0.2) | 9 (0.9) |
| Angina, n (%) | 5 (1.0) | 0 | 5 (0.5) |
| Higher education or degree-level qualifications, n (% of respondents)* | 160 (32.7) | 196 (41) | 356 (36.8) |
| Clinic BP phenotypes, mm Hg | | | |
| Clinic SBP, mean (SD) | 132.8 (19.2) | 120.1 (15.1) | 126.5 (18.4) |
| Clinic DBP, mean (SD) | 82.9 (11.2) | 73.5 (8.9) | 78.2 (11.2) |
| Clinic BP ≥140/90, n (%) | 191 (38.3) | 61 (12.3) | 252 (25.3) |
| Ambulatory BP phenotypes, mm Hg | | | |
| 24-Hour mean SBP, mean (SD) | 121.1 (12.3) | 117.4 (8.8) | 119.2 (10.8) |
| 24-Hour mean DBP, mean (SD) | 75.0 (8.3) | 69.1 (5.8) | 72.0 (7.7) |
| Mean daytime SBP, mean (SD) | 127.4 (13.4) | 122.6 (9.4) | 125.0 (11.8) |
| Mean daytime DBP, mm Hg, mean (SD) | 80.2 (9.0) | 74.0 (6.6) | 77.1 (8.4) |
| Mean nighttime SBP, mm Hg, mean (SD) | 112.8 (12.0) | 110.7 (9.3) | 111.7 (10.8) |
| Mean nighttime DBP, mm Hg, mean (SD) | 68.2 (8.4) | 62.6 (6.2) | 65.4 (7.9) |

*Educational level data available for 968 of the 996 subjects.
†Alcohol intake data available for 991 subjects.

using HBAT.[21] The input values for HBAT were the residuals from a normal linear regression correcting for age and sex as covariates and adjusting for treatment effects using a nonparametric algorithm.[4] Probability values were inferred by a permutation method described by Horvath et al,[21] using up to 100 000 Monte Carlo samples.

Further details of the statistical analyses and adjustment methods for antihypertensive treatment are available in the Data Supplement.

## Results

### GRAPHIC Study Families

Table 1 shows the characteristics of the 996 subjects (from 250 families) included in the analyses. Mean 24-hour ambulatory SBP (SD) was 119.2 (10.8), and mean ambulatory DBP was 72.0 (7.7). There were significant correlations between the different ambulatory BP phenotypes and also between these phenotypes and clinic BP (Data Supplement Table I). A history of hypertension was reported by 136 (13.7%) subjects, of whom 63 (6.3%) were currently receiving antihypertensive treatment.

### Allele Frequencies and LD

Table 2 summarizes the genomic location, allele frequency, and Hardy-Weinberg tests for the 9 *WNK1* SNPs analyzed. None of the SNPs showed statistically significant deviation from Hardy-Weinberg equilibrium. Strong, pairwise LD was observed between the intragenic SNPs from intron 1 to intron 26 (Data Supplement Figure I). The *WNK4* SNP (1156666G→A) had a minor allele frequency of 11.1% and did not deviate from Hardy-Weinberg equilibrium ($P=0.62$).

### Heritability

The estimated proportion of the BP variance attributable to additive polygenic effects (ie, the narrow-sense heritability, or $h^2_N$) was 63.4% (95% CI, 52.3% to 73.3%) for mean 24-hour SBP and 67.9% (95% CI, 57.5% to 77.2%) for mean 24-hour DBP.

### Primary Association Analyses

Of the 9 *WNK1* SNPs, 4 exhibited a significant association ($P<0.05$) with mean 24-hour SBP and 5 with mean 24-hour DBP (Table 3). Furthermore, 2 of these SNPs (rs880054 in intron 10 and rs2301880 in intron 23) showed highly significant associations ($P<0.005$) with mean 24-hour SBP and DBP, respectively. In addition, rs765250 in intron 1 exhibited a highly significant association ($P<0.005$) with mean nighttime SBP. Under an additive genetic model, the coefficient for each SNP (Table 3) may be interpreted as the mean increase in BP (in mm Hg) associated with each additional copy of the minor allele. For example, the most common SNP rs880054 was associated with a mean reduction in 24-hour SBP of 1.37 mm Hg per copy of the *C* (minor) allele (95% CI,

**TABLE 2. Description of the *WNK1* SNPs Genotyped in the GRAPHIC Study**

| *WNK1* SNP* | Chromosome 12 Position† | *WNK1* Position | Alleles‡ | n§ | Minor-Allele Frequency (Parents)‖ | Hardy-Weinberg Test‖ Pearson's $\chi^2$ (1 *df*) | P |
|---|---|---|---|---|---|---|---|
| rs1468326 | 727762 | 5′ region | C/A | 987 | 11.3% | 0.023 | 0.88 |
| rs2369402 | 748925 | Intron 1 | *G/A* | 990 | 22.1% | 0.046 | 0.83 |
| rs765250 | 778544 | Intron 1 | *T/C* | 990 | 31.1% | 0.19 | 0.66 |
| rs2286007 | 841552 | Exon 8 | *C/T* | 995 | 8.5% | 1.87 | 0.18 |
| rs880054 | 858819 | Intron 10 | *T/C* | 993 | 43.8% | 1.37 | 0.24 |
| rs956868 | 861173 | Exon 13 | *C/A* | 992 | 14.2% | 3.39 | 0.065 |
| rs953361 | 872068 | Intron 22 | *C/T* | 987 | 39.0% | 1.40 | 0.24 |
| rs2301880 | 874098 | Intron 23 | *C/T* | 991 | 26.1% | 0.55 | 0.46 |
| rs2286028 | 885730 | Intron 26 | *G/C* | 987 | 17.6% | 0.32 | 0.57 |

*dbSNP accession number.
†Chromosome 12 position in base-pairs (source: HapMap Web site, http://www.hapmap.org/).
‡Major/minor alleles shown.
§Number of subjects with genotype data available for analysis.
‖Minor allele frequency and Hardy-Weinberg tests relate to the parents (parental generation).

2.45-mm Hg reduction to a 0.23-mm Hg reduction). We found no evidence of modification of the effect of any of the *WNK1* SNPs on mean 24-hour SBP or DBP by sex. The *WNK4* SNP (1156666G→A) was not associated with any of the BP phenotypes.

## Secondary Association Analyses

Secondary analyses were carried out to examine (1) the association between *WNK1* variants and clinic BP; (2) the effect of different genetic models, and (3) the impact of adjustments for additional covariates. Three *WNK1* SNPs were associated ($P<0.05$) with clinic SBP and 5 with clinic DBP ($P=0.00087$ for association between rs880054 and clinic DBP; Data Supplement Table II). Significant associations were observed between *WNK1* SNPs and mean 24-hour SBP and DBP under different genetic models (Data Supplement Tables III to V). The data are consistent with a codominant effect for the majority of SNPs, but a dominant effect cannot be ruled out. Significant associations between the *WNK1* SNPs and mean 24-hour SBP and DBP were also evident after correcting for body mass index in addition to age and sex (Data Supplement Table VI) and also after including a range of other covariates, such as smoking, alcohol intake, history of hypercholesterolemia, and educational level (Data Supplement Table VII).

## Haplotype Analyses of *WNK1*: Joint Linkage and Association

Fifteen haplotypes with a frequency of 1% or greater were identified, and 4 of these showed significant evidence ($P<0.05$) of an association in the presence of linkage with mean 24-hour SBP and/or mean 24 hour DBP (Table 4). Of these, 2 haplotypes were common: h2 'CGTCTCTC' and h4 'CACCCCCG' (frequencies of 0.158 and 0.126, respectively). Haplotype h2 was associated with a significant rise in mean 24-hour DBP, whereas h4 was associated with a fall in both mean 24-hour SBP and mean 24-hour DBP. Another haplotype, h7 'CGCCTCCG' (frequency, 0.040), was associated with a rise in BP across all BP phenotypes, with significant associations with 5 of the 6 ambulatory BP

**TABLE 3. Estimates of the Effects of the *WNK1* SNPs and *WNK4* SNP on Ambulatory SBP and DBP Phenotypes**

| | Mean 24-Hour SBP | | Mean Daytime SBP | | Mean Nighttime SBP | | Mean 24-Hour DBP | | Mean Daytime DBP | | Mean Nighttime DBP | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SNP | Coefficient (95% CI) | P | Coefficient (95% CI) | P | Coefficient (95% CI) | P | Coefficient (95% CI) | P | Coefficient (95% CI) | P | Coefficient (95% CI) | P |
| *WNK1* | | | | | | | | | | | | |
| rs1468326 | −0.94 (−2.65 to 0.75) | 0.27 | −0.95 (−2.85 to 0.89) | 0.32 | −1.08 (−2.84 to 0.64) | 0.22 | −0.87 (−2.02 to 0.33) | 0.15 | −1.02 (−2.30 to 0.26) | 0.12 | −0.73 (−1.84 to 0.44) | 0.21 |
| rs2369402 | **−1.42 (−2.75 to −0.06)** | **0.037** | −1.42 (−2.87 to −0.01) | 0.053 | **−1.45 (−2.77 to −0.09)** | **0.031** | **−0.98 (−1.87 to −0.04)** | **0.036** | **−1.01 (−2.0 to −0.00)** | **0.044** | **−0.90 (−1.77 to −0.01)** | **0.046** |
| rs765250 | **−1.50 (−2.69 to −0.24)** | **0.016** | −1.16 (−2.52 to 0.16) | 0.085 | **−1.81 (−2.91 to −0.69)** | **0.0015** | −0.84 (−1.70 to 0.07) | 0.060 | −0.77 (−1.68 to 0.14) | 0.098 | **−0.91 (−1.71 to −0.09)** | **0.030** |
| rs2286007 | −0.80 (−2.71 to 1.16) | 0.42 | −0.42 (−2.51 to 1.62) | 0.70 | −1.16 (−3.04 to 0.68) | 0.23 | −1.05 (−2.34 to 0.23) | 0.11 | −0.99 (−2.37 to 0.36) | 0.16 | −0.97 (−2.26 to 0.35) | 0.14 |
| rs880054 | **−1.37 (−2.45 to −0.23)** | **0.015** | **−1.48 (−2.60 to −0.31)** | **0.012** | **−1.30 (−2.44 to −0.27)** | **0.019** | **−1.14 (−1.93 to −0.38)** | **0.0041** | **−1.18 (−2.02 to −0.33)** | **0.0060** | **−1.00 (−1.78 to −0.20)** | **0.012** |
| rs956868 | 1.46 (−0.15 to 3.14) | 0.081 | 1.59 (−0.16 to 3.24) | 0.067 | 1.40 (−0.23 to 3.03) | 0.092 | 0.67 (−0.43 to 1.79) | 0.23 | 0.89 (−0.29 to 2.07) | 0.14 | 0.51 (−0.57 to 1.60) | 0.36 |
| rs953361 | 1.01 (−0.11 to 2.07) | 0.072 | 0.88 (−0.29 to 2.05) | 0.14 | 1.12 (−0.03 to 2.28) | 0.059 | **0.89 (0.10 to 1.67)** | **0.026** | **0.86 (−0.02 to 1.69)** | **0.049** | **0.91 (0.18 to 1.70)** | **0.017** |
| rs2301880 | **−1.78 (−3.00 to −0.55)** | **0.0045** | **−1.62 (−3.0 to −0.34)** | **0.017** | **−2.0 (−3.24 to −0.81)** | **0.0012** | **−1.07 (−1.85 to −0.24)** | **0.0099** | **−1.05 (−2.01 to −0.13)** | **0.029** | **−1.06 (−1.95 to −0.21)** | **0.018** |
| rs2286028 | 0.99 (−0.45 to 2.38) | 0.17 | 1.12 (−0.45 to 2.66) | 0.16 | 0.93 (−0.53 to 2.39) | 0.19 | **1.00 (0.04 to 1.99)** | **0.039** | **1.08 (0.05 to 2.14)** | **0.041** | **0.98 (0 to 2.00)** | **0.050** |
| *WNK4* | | | | | | | | | | | | |
| (1156666G>A) | 0.09 (−1.67 to 1.77) | 0.92 | 0.12 (−1.74 to 1.93) | 0.90 | −0.04 (−1.70 to 1.67) | 0.96 | 0.10 (−1.12 to 1.29) | 0.87 | 0.16 (−1.15 to 1.40) | 0.81 | −0.01 (−1.22 to 1.23) | 0.98 |

All analyses took full account of familial relationships and were adjusted for age, age$^2$, and sex as covariates. The coefficients are shown under an additive genetic model and may be interpreted as a per-allele effect. Significant results ($P<0.05$) are shown in boldface type.

**TABLE 4. Joint Linkage and Association Analysis of Haplotypes in *WNK1* With Ambulatory SBP and DBP Phenotypes***

| Haplotype | | | | Mean 24-Hour SBP | | Mean Daytime SBP | | Mean Nighttime SBP | | Mean 24-Hour DBP | | Mean Daytime DBP | | Mean Nighttime DBP | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alleles | Name | Frequency | n† | *Z*‡ | *P* | *Z* | *P* | *Z* | *P* | *Z* | *P* | *Z* | *P* | *Z* | *P* |
| CGTCTCTG | h1 | 0.175 | 120 | 0.361 | 0.73 | 0.240 | 0.81 | 0.494 | 0.62 | 0.726 | 0.47 | 0.911 | 0.35 | 0.333 | 0.73 |
| *CGTCTCTC* | h2 | 0.158 | 106 | −0.512 | 0.61 | −0.473 | 0.64 | −0.498 | 0.62 | **2.291** | **0.021** | **1.966** | **0.052** | **1.962** | **0.048** |
| *CGTCCCCG* | h3 | 0.139 | 98 | 0.206 | 0.84 | 0.233 | 0.81 | 0.049 | 0.96 | −1.403 | 0.16 | −1.207 | 0.23 | −1.555 | 0.12 |
| *CACCCCCG* | h4 | 0.126 | 84 | **−2.264** | **0.025** | **−2.230** | **0.023** | −1.854 | 0.064 | **−2.159** | **0.032** | **−2.142** | **0.032** | −1.512 | 0.14 |
| *CGTCTACG* | h5 | 0.126 | 80 | −0.663 | 0.51 | −0.843 | 0.40 | −0.271 | 0.78 | −1.287 | 0.20 | −1.146 | 0.26 | −1.103 | 0.27 |
| *CACTCCCG* | h6 | 0.074 | 58 | 1.847 | 0.059 | **1.980** | **0.047** | 1.391 | 0.17 | 1.210 | 0.22 | 1.030 | 0.30 | 1.245 | 0.21 |
| *CGCCTCCG* | h7 | 0.040 | 32 | **2.248** | **0.022** | **2.450** | **0.014** | 1.616 | 0.11 | **2.707** | **0.0053** | **2.576** | **0.0082** | **2.357** | **0.014** |
| *AGTCCCCG* | h8 | 0.024 | 21 | −1.592 | 0.11 | −1.010 | 0.32 | −1.646 | 0.095 | −0.471 | 0.64 | −0.086 | 0.94 | −0.437 | 0.66 |
| *CGCCCCCG* | h9 | 0.023 | 23 | −1.749 | 0.083 | −1.161 | 0.25 | **−2.099** | **0.038** | **−2.117** | **0.032** | −1.683 | 0.091 | **−1.961** | **0.047** |
| *AGCCTCCG* | h10 | 0.016 | 15 | 0.352 | 0.73 | 0.450 | 0.65 | 0.089 | 0.91 | −0.148 | 0.89 | −0.037 | 0.97 | −0.246 | 0.82 |
| *CGTCCCTG* | h11 | 0.016 | 16 | 0.432 | 0.67 | 0.422 | 0.68 | 0.488 | 0.63 | −1.381 | 0.17 | −1.408 | 0.16 | −0.876 | 0.39 |
| AGTCTCTC | h12 | 0.016 | 16 | 0.055 | 0.95 | −0.402 | 0.70 | 0.615 | 0.55 | −1.213 | 0.23 | −1.46 | 0.15 | −0.283 | 0.78 |
| *AGTCTCTG* | h13 | 0.014 | 14 | 0.438 | 0.68 | 0.393 | 0.70 | 0.386 | 0.69 | −0.470 | 0.66 | −0.720 | 0.48 | −0.079 | 0.94 |
| *AGCCCCCG* | h14 | 0.012 | 10 | 1.701 | 0.077 | **2.038** | **0.034** | 0.712 | 0.51 | 0.675 | 0.53 | 1.286 | 0.22 | −0.235 | 0.81 |
| *AACCCCCG* | h15 | 0.010 | 10 | 0.535 | 0.60 | −0.143 | 0.89 | 0.978 | 0.34 | 0.695 | 0.50 | 0.332 | 0.76 | 0.602 | 0.54 |
| Global (multihaplotype) test (*P*)§ | | | | | 0.058 | | 0.14 | | 0.12 | | **0.011** | | 0.064 | | 0.086 |

*Family-based association tests for haplotypes were used for the analysis, implemented with the HBAT extension of the FBAT toolkit.[21] Significant results ($P<0.05$) are shown in boldface type.

†n Indicates number of informative families.

‡A negative *Z* score indicates a haplotype that reduces BP, and a positive *Z* score, a haplotype that increases BP.

§In addition to the haplotype-specific test, a more conservative global test of joint linkage and association across all haplotypes with at least 10 informative families is shown.

phenotypes ($P=0.0053$ for mean 24-hour DBP). In addition, h9 'CGCCCCCG' (frequency, 0.023) was associated with a fall in BP across all phenotypes, with significant findings for 3 BP phenotypes. A multihaplotype (global) test of linkage and association was statistically significant for mean 24-hour DBP ($P=0.011$) but of only borderline significance for mean 24-hour SBP ($P=0.058$). Of interest, the 2 haplotypes with the most discordant estimated effects were h4 and h7. These haplotypes differed in the alleles of rs2369402 and rs880054, which themselves showed significant associations with BP (Table 3).

## Discussion

We report statistically significant associations of common SNPs spanning introns 1 to 26 of the *WNK1* gene with ambulatory SBP and DBP in the general population. All SNPs that had a minor-allele frequency of >0.15 showed significant association with at least 1 ambulatory BP phenotype.

Any reported genetic association should be interpreted with caution until it is replicated. However, the following evidence supports a genuine rather than a chance or artifactual association between *WNK1* variants and BP. First, there is a priori evidence of involvement of the gene in a monogenic form of hypertension.[6] Second, power calculations a priori and post hoc suggest that this study was adequately powered (see Data Supplement). Third, the haplotype analyses support the results of those based on the individual SNPs. Although the GLMM-based approach is robust to stratification and admixture,[22] the haplotype analyses provide further reassurance that these findings were not solely attributable to population substructure.[21] Fourth, although genetic association studies should not be subject to confounding by lifestyle factors,[23] significant associations were still noted between *WNK1* SNPs and mean 24-hour SBP and DBP after correction for a range of covariates.

Although we did not apply a Bonferroni correction, our findings cannot simply be explained by multiple testing. We tested a limited number of *WNK1* SNPs in strong LD and found at least 1 significant association for each common SNP. Furthermore, 2 associations with mean 24-hour BP phenotypes and 2 associations with nighttime BP phenotypes were highly significant ($P<0.005$), and even the low-power global test supported significant linkage and association of the *WNK1* haplotypes with mean 24-hour DBP ($P=0.011$).

The GRAPHIC study adopted a design and analytic strategy that optimized the ability to detect genetic determinants of BP. First, we studied BP as a continuous trait. This is a powerful approach, particularly when measurement error is minimized. We therefore used ambulatory BP monitoring, the most precise noninvasive measure of usual BP that is available. Second, through participating family practices, we generated a study population that was representative of the English population in terms of BP and age-appropriate prevalence and treatment of hypertension.[24,25] This sampling strategy not only allows generalizability of the findings but also avoids loss of power with adjustment for ascertainment bias.[26] Third, we used censored normal[19] and nonparametric[4]

approaches to adjust for the effects of antihypertensive therapy. These methods avoid the bias and loss in power that arise from inappropriate correction for treatment effects.[19] Fourth, the nuclear family–based design of the GRAPHIC study permitted the study of variance components and heritability and is robust to population stratification. Our estimates of heritability are consistent with other studies that have minimized the measurement error for BP.[3,4]

The effects of the *WNK1* variants on BP may seem modest. For example, the proportion of the additive polygenic variance in mean 24-hour SBP explained by SNP rs2301880 is ≈1.4% (ie, 0.9% of the total variance). However, the estimated magnitude of these effects is entirely consistent with what might be expected for a complex genetic trait. Importantly, even a 2-mm Hg-lower usual SBP is associated with an ≈10% fall in stroke mortality and a 7% reduction in mortality from ischemic heart disease or other vascular causes in middle age.[27] SNP rs2301880 exhibited a minor-allele frequency of 26% and a per-allele reduction in SBP of 1.78 mm Hg, a difference of >3.5 mm Hg between *TT* and *CC* homozygotes. The magnitude of the estimated effect is comparable to that seen with a modest reduction in dietary sodium.[28] These observations highlight the potential public health importance of our findings.

No other study to date has reported an association between *WNK1* variants and BP in a population-based sample. Kokubo et al[11] found no association between *WNK1* SNPs and clinic SBP or DBP in a population of 771 hypertensives and 1047 controls selected from within the Suita cohort in Japan. Recently, the BRIGHT study showed nominal evidence of an association between *WNK1* SNP rs1468326 and SBP and DBP in a family study of extremely hypertensive subjects.[13]

Only 1 common SNP has been reported in *WNK4* in white European subjects.[10] This is in intron 10 (1156666G>A), and there are conflicting reports of its association with hypertension.[10,12] We observed allele frequencies of this SNP similar to those in previous studies[10,12] but found no association with either ambulatory or clinic BP in our population. We have also confirmed that several other SNPs reported in *WNK4* in dbSNP are not polymorphic in white Europeans and have also undertaken an SNP screening project of the promoter and functional domains of *WNK4* by direct sequencing of 20 individuals with divergent *WNK1* haplotypes (S.N. and P.B.M., unpublished data). No novel SNPs were found. Our results are consistent with those of Erlich et al[10] and suggest that *WNK4* does not contain common polymorphisms in white Europeans.

Our study shows the potential importance of *WNK1* in BP regulation in humans. This is the first study to show that a gene causing a monogenic form of hypertension plays a significant role in BP regulation in the general population. Given the function of *WNK1* in renal sodium and potassium homeostasis, it will be important to investigate whether there are interactions between *WNK1* variants with modifiable environmental exposures, such as dietary salt intake. If such interactions are found, then the modification of such exposures may lead to a disproportionate effect in certain population subgroups with important health consequences. Even greater public health benefits might be realized if a more robust understanding of the biological pathways through which *WNK1* exerts its effects leads to identification of an intermediate phenotype that might be amenable to modification in whole populations.[23] In addition, if knowledge of the role of *WNK1* in BP regulation were to lead to the development of antihypertensive drugs with improved efficacy or acceptability, then substantial improvements in BP control in the treated hypertensive population may be achieved. Given that thiazide diuretics cause a particularly large fall in BP in PHAII patients,[9] pharmacogenetic studies to establish whether or not the efficacy, side effects, and acceptability of different classes of antihypertensive agents vary with polymorphisms in the *WNK1* gene may become relevant if our findings are confirmed in other studies.

## Limitations

We studied a relatively healthy, young to middle-aged white European population. Although BP values were broadly representative of the English general population,[25] the generalizability of our findings to older age groups, less healthy individuals, and different ethnic groups needs to be established. Furthermore, we have not identified the causal variants in *WNK1* that are responsible for the effect on BP. *WNK1* is a relatively large gene, and although we assumed that the 8 tSNPs lie in a single block, it is also possible that the tSNPs could span >1 haplotype block. The SNPs showing a significant association spanned almost the whole *WNK1* gene (from intron 1 to intron 26), and all were intronic and unlikely to be functional. Therefore, further investigations are required to pinpoint specific regions of the *WNK1* gene that harbor functional genetic variants. Finally, the mechanisms by which any causal variants in *WNK1* affect BP need to be elucidated.

In summary, common variants in the *WNK1* gene contribute to BP variation in the general population. The findings provide a basis to identify functional variants of *WNK1,* elucidate any interactions of these variants with dietary intake or the response to antihypertensive drugs, and determine their impact on cardiovascular morbidity and mortality.

## Acknowledgments

The GRAPHIC study is funded through a British Heart Foundation program grant. This work was also supported by the Wellcome Trust Functional Genomics Initiative in Cardiovascular Genetics; an MRC cooperative grant on variability, instability, and pathology of the human genome; and an MRC clinical training fellowship (to Dr Tobin). Professor Samani holds a British Heart Foundation Chair of Cardiology. We gratefully acknowledge the support of the participating families and the general practitioners and staff at the Melton, Oakham, Broughton Astley, Wigston, Kibworth, and Market Harborough practices for their assistance with the recruitment of families. Finally, we thank Kim Mason, Jean Hudson, and Louise Goddard for their clerical support; Leighton Crosbee, Annette Brindle, Danny Kirby, and Rebecca Quinn for computing support and database development; and Chris Wallace for helpful discussions.

## References

1. National Institutes of Health. *JNC 7 Express: the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure.* Bethesda, Md: National Institutes of Health; 2003.

2. Murray CJ, Lauer JA, Hutubessy RC, Niessen L, Tomijima N, Rodgers A, Lawes CM, Evans DB. Effectiveness and costs of interventions to lower systolic blood pressure and cholesterol: a global and regional analysis on reduction of cardiovascular-disease risk. *Lancet*. 2003;361: 717–725.
3. Kotchen TA, Kotchen JM, Grim CE, George V, Kaldunski ML, Cowley AW, Hamet P, Chelius TH. Genetic determinants of hypertension: identification of candidate phenotypes. *Hypertension*. 2000;36:7–13.
4. Levy D, DeStefano AL, Larson MG, O'Donnell CJ, Lifton RP, Gavras H, Cupples LA, Myers RH. Evidence for a gene influencing blood pressure on chromosome 17: genome scan linkage results for longitudinal blood pressure phenotypes in subjects from the Framingham Heart Study. *Hypertension*. 2000;36:477–483.
5. Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. *Cell*. 2001;104:545–556.
6. Wilson FH, Disse-Nicodeme S, Choate KA, Ishikawa K, Nelson-Williams C, Desitter I, Gunel M, Milford DV, Lipkin GW, Achard JM, Feely MP, Dussol B, Berland Y, Unwin RJ, Mayan H, Simon DB, Farfel Z, Jeunemaitre X, Lifton RP. Human hypertension caused by mutations in WNK kinases. *Science*. 2001;293:1107–1112.
7. Wilson FH, Kahle KT, Sabath E, Lalioti MD, Rapson AK, Hoover RS, Hebert SC, Gamba G, Lifton RP. Molecular pathogenesis of inherited hypertension with hyperkalemia: the Na-Cl cotransporter is inhibited by wild-type but not mutant WNK4. *Proc Natl Acad Sci U S A*. 2003;100: 680–684.
8. Yang CL, Angell J, Mitchell R, Ellison DH. WNK kinases regulate thiazide-sensitive Na-Cl cotransport. *J Clin Invest*. 2003;111:1039–1045.
9. Mayan H, Vered I, Mouallem M, Tzadok-Witkon M, Pauzner R, Farfel Z. Pseudohypoaldosteronism type II: marked sensitivity to thiazides, hypercalciuria, normomagnesemia, and low bone mineral density. *J Clin Endocrinol Metab*. 2002;87:3248–54.
10. Erlich PM, Cui J, Chazaro I, Farrer LA, Baldwin CT, Gavras H, DeStefano AL. Genetic variants of WNK4 in whites and African Americans with hypertension. *Hypertension*. 2003;41:1191–1195.
11. Kokubo Y, Kamide K, Inamoto N, Tanaka C, Banno M, Takiuchi S, Kawano Y, Tomoike H, Miyata T. Identification of 108 SNPs in TSC, WNK1, and WNK4 and their association with hypertension in a Japanese general population. *J Hum Genet*. 2004;49:507–515.
12. Speirs HJ, Morris BJ. *WNK4* intron 10 polymorphism is not associated with hypertension. *Hypertension*. 2004;43:766–768.
13. Newhouse SJ, Wallace C, Dobson R, Mein C, Pembroke J, Farrall M, Clayton D, Brown M, Samani N, Dominiczak A, Connell JM, Webster J, Lathrop GM, Caulfield M, Munroe PB. Haplotypes of the WNK1 gene associate with blood pressure variation in a severely hypertensive population from the British Genetics of Hypertension (BRIGHT) study. *Hum Mol Genet*. 2005;14:1805–1814.
14. Stram DO, Haiman CA, Hirschhorn JN, Altshuler D, Kolonel LN, Henderson BE, Pike MC. Choosing haplotype-tagging SNPS based on unphased genotype data using a preliminary sample of unrelated subjects with an example from the Multiethnic Cohort Study. *Hum Hered*. 2003;55:27–36.
15. O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet*. 1998; 63:259–266.
16. JLIN: Java LINkage disequilibrium plotter. Available at http://www.genepi.com.au/jlin; last accessed February 11, 2005.
17. Burton PR, Tiller KJ, Gurrin LC, Cookson WO, Musk AW, Palmer LJ. Genetic variance components analysis for binary phenotypes using generalized linear mixed models (GLMMs) and Gibbs sampling. *Genet Epidemiol*. 1999;17:118–140.
18. Spiegelhalter D, Thomas A, Best N. *WinBUGS Version 1.4 User Manual*. Cambridge, England: MRC Biostatistics Unit; 2003.
19. Tobin MD, Sheehan NA, Scurrah KJ, Burton PR. Adjusting for treatment effects in studies of quantitative traits: antihypertensive therapy and systolic blood pressure. *Stat Med*. 2005;24:2911–2935.
20. Burton PR, Gurrin LC, Campbell MJ. Clinical significance not statistical significance: a simple Bayesian alternative to p values. *J Epidemiol Community Health*. 1998;52:318–323.
21. Horvath S, Xu X, Lake SL, Silverman EK, Weiss ST, Laird NM. Family-based tests for associating haplotypes with general phenotype data: application to asthma genetics. *Genet Epidemiol*. 2004;26:61–69.
22. Allison DB, Heo M, Kaplan N, Martin ER. Sibling-based tests of linkage and association for quantitative traits. *Am J Hum Genet*. 1999;64: 1754–1763.
23. Davey Smith G, Ebrahim S. 'Mendelian randomisation': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol*. 2003;32:1–22.
24. Primatesta P, Brookes M, Poulter NR. Improved hypertension management and control: results from the health survey for England 1998. *Hypertension*. 2001;38:827–932.
25. Joint Health Surveys Unit. *Health Survey for England 2003. Volume 2: Risk Factors for Cardiovascular Disease*. London: The Stationery Office; 2004.
26. Burton PR. Correcting for non-random ascertainment in generalized linear mixed models (GLMMs) fitted using Gibbs sampling. *Genet Epidemiol*. 2003;24:24–35.
27. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet*. 2002;360:1903–1913.
28. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, Obarzanek E, Conlin PR, Miller ER 3rd, Simons-Morton DG, Karanja N, Lin PH, Group DA-SCR. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet; DASH-Sodium Collaborative Research Group. *N Engl J Med*. 2001; 344:3–10.